Appeal decision

Appeal No. 2017-13795

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The case of appeal against the examiner's decision of refusal of Japanese Patent Application No. 2016-117740 "System and method for sequence manipulation and engineering of optimized guide compositions" [the application published on September 15, 2016, Japanese Unexamined Patent Application Publication No. 2016-165307] has resulted in the following appeal decision:

Conclusion

The appeal of the case was groundless.

Reason
No. 1 History of the procedures

The present application is a divisional application filed on June 14, 2016 from Japanese Patent Application No. 2015-547573 filed on December 12, 2013 with an international filing date (priority claims under the Paris Convention: December 12, 2012, U.S.A.; January 2, 2013, U.S.A.; January 30, 2013, U.S.A; February 25, 2013, U.S.A.; March 15, 2013, U.S.A.; March 28, 2013, U.S.A.; April 20, 2013, U.S.A.; May 6, 2013, U.S.A.; May 28, 2013, June 17, 2013, U.S.A.; for March 15, 2013 and June 17, 2013, priority claims were made for two basic applications with the same priority date respectively), and the history of the procedure is as follows:

August 3, 2016: Notification of reasons for refusal
February 8, 2017: Submission of written opinion and written correction of mistranslation
April 28, 2017: The examiner's decision of refusal
September 15, 2017: Submission of written demand for trial
November 1, 2017: Submission of written amendment and a supplemental statement against the written demand for trial

No. 2 The Invention

While it is recognized that the inventions according to claims 1 to 21 of the present application are specified by the matters described in claims 1 to 21 of the scope of claims after Correction of Mistranslation by Written Correction of Mistranslation submitted on February 8, 2017, the invention according to claim 1 (hereinafter, the "Invention") is as shown below.

"[Claim 1]

An engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR-associated (Cas) (CRISPR-Cas) vector system comprising one or more vectors comprising
a) a first regulatory element operably linked to a nucleotide sequence encoding CRISPR-Cas system polynucleotide sequence comprising a guide sequence, a tracrRNA sequence, and a tracr mate sequence, wherein the guide sequence hybridizes to at least one target sequence of polynucleotide loci in a eukaryotic cell.
b) a second regulatory element operably linked to a nucleotide sequence encoding a Type II Cas9 protein, and
c) a recombinant template,
wherein components (a), (b), and (c) are located on the same or different vectors of the system, and the system further comprises one or more nuclear localization signals (NLSs) expressed together with the nucleotide sequence that encodes the Cas9 protein, whereby the guide sequence targets the one or more polynucleotide loci in the eukaryotic cell, and the Cas9 protein cleaves the one or more polynucleotide loci, whereby the sequence of the one or more polynucleotide loci are modified.

No. 3 Reasons for refusal stated in the examiner's decision

Reasons for refusal stated in the examiner's decision are shown below.

1. Since inventions according to claims 1 to 21 of the present application are the same as inventions described in the description, the scope of claims and drawings of the international application having the international filing date of the following prior application 1 internationally published after the filing of the present application, which international application is a foreign language patent application (excluding those deemed to have been withdrawn under the provisions of Article 184-4(3) of the Patent Act) filed before the filing date of the present application, and, in addition, the inventor of the present application is not the same as the inventor of the above foreign language patent application before the filing of the present application and, as of the filing of this application, the applicant is not the same as the applicant of the above foreign language patent application, Appellant should not be granted a patent for the inventions according to claims 1 to 16 of the present application under the provisions of Article 29-2 of the Patent Act (see Article 184-13 of the Patent Act).

2. Since the inventions according to claims 1 to 8 and 12 to 21 of the present application could have easily been invented by a person skilled in the art based on the invention described in the following Cited Document 2 that is a publication distributed before the priority date of the present application and well-known arts, Appellant should not be granted a patent for the inventions according to claims 1 to 16 of the present application under the provisions of Article 29-2 of the Patent Act.

Prior Application 1: PCT/US 2013/073307 (international publication No. 2014/089290, National publication of International Patent Application No. 2016-502840)
Cited Document 2: Science, Aug 2012, Vol. 337, pp. 816-821, Supplementary Materials

No. 4 Reason 1 (Article 29-2 of the Patent Act)

1. Description in the originally attached description, etc. of Prior Application 1

The description, the scope of claims, and drawings of a foreign language patent application cited by the examiner's decision (excluding those deemed to have been withdrawn under the provisions of Article 184-4(3) of the Patent Act) of which first priority date under the Paris Convention (hereinafter, "the first priority date of the prior application 1") is December 6, 2012 before the first priority date of the present application (December 12, 2012), which foreign language patent application is an international application having the international filing date of the following prior application 1 (PCT/US2013/073307 (International publication No. 2014/089290, National publication of International Patent Application No. 2016-502840)) internationally published after the filing of the present application (hereinafter, the "the originally attached description, etc. of Prior Application 1") describe the following matters. In addition, together with it, matters similar to those matters are also described in U.S. Patent Application (61/734,256) that is the basis for the first priority date of Prior Application 1. Since it is written in English language, described matters of and the source of citation in National publication of International Patent Application No. 2016-502840 that is the national publication of Prior Application 1 are shown as a translation (underline added by the body).

(1-1) "[Claim 13] A method for modifying a chromosomal sequence in a eukaryotic cell or embryo, the method comprising: a) introducing into the eukaryotic cell or embryo (i) at least one RNA- guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide; and b) culturing the eukaryotic cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified.
[Claim 14] The method of claim 13, wherein the RNA-guided endonuclease is derived from a Cas9 protein."

(1-2) "[0004]
...
In other embodiments, the nucleic acid sequence encoding the RNA-guided endonuclease can be operably linked to a promoter control sequence, and optionally, can be part of a vector. In other embodiments, a vector comprising a sequence encoding the RNA-guided endonuclease, which can be operably linked to a promoter control sequence, can also comprise a sequence encoding a guide RNA, which can be operably linked to a promoter control sequence.

(1-3) "[0014]
In one embodiment, the RNA-guided endonuclease is derived from a type II CRISPR/Cas system. In specific embodiments, the RNA-guided endonuclease is derived from a Cas9 protein."

(1-4) "[0022]
In certain embodiments, the RNA-guided endonuclease may be part of a protein-RNA complex comprising a guide RNA. The guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, a specific protospacer sequence at 5' end of the guide RNA base pairs."

(1-5) "[0060]
...
In embodiments in which the optional donor polynucleotide is present, the donor sequence in the donor polynucleotide can be exchanged with or integrated into the chromosomal sequence at the targeted site during repair of the double-stranded break. For example, in embodiments in which the donor sequence is flanked by upstream and downstream sequences having substantial sequence identity with upstream and downstream sequences, respectively, of the targeted site in the chromosomal sequence, the donor sequence can be exchanged with or integrated into the chromosomal sequence at the targeted site during repair mediated by a homology-directed repair process."

(1-6) "[0066]

(b) Guide RNA

The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA. A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific target site, at which site the 5' end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence.

[0067]

Each guide RNA comprises three regions: a first region at the 5' end that is complementary to the target site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific target site. The second and third regions of each guide RNA can be the same in all guide RNAs."

(1-7) "[0085]

A donor polynucleotide that comprises upstream and downstream sequences that have sequence similarity with the target chromosome sequence could be straight chain or circular. In an embodiment in which the donor polynucleotide is circular, it could be a part of a vector. For example, the vector could be a plasmid vector.

(1-8) "[0145]

Example 3: Preparation of Donor Polynucleotide to Monitor Genome Modification

Targeted integration of a GFP protein into the N terminus of PPP1 R12C was used to monitor Cas9-mediated genome modification. To mediate integration by homologous recombination, a donor polynucleotide was prepared. The AAVS1 -GFP DNA donor contained a 5' (1 185 bp) AAVS1 locus homologous arm, an RNA splicing receptor, a turbo GFP coding sequence, a 3' transcription terminator, and a 3' (1217 bp) AAVS1 locus homologous arm. Table 5 presents the sequences of the RNA splicing receptor and the GFP coding sequence, followed by the 3' transcription terminator. Plasmid DNA was prepared by using GenElute Endotoxin-Free Plasmid Maxiprep Kit (Sigma).

...

[0147]

Targeted gene integration will result in a fusion protein between the first 107 amino acids of the PPP1 R12C and the turbo GFP. The expected fusion protein contains the first 107 amino acid residues of PPP1 R12C (highlighted in grey) from RNA splicing between the first exon of PPP1 R12C and the engineered splice receptor (see Table 6)."

(1-9) "[0149]

Example 4: Cas9-Mediated Targeted Integration

Transfection was performed on human K562 cells. The K562 cell line was obtained from American Type Culture Collection (ATCC) and grown in Iscove's Modified Dulbecco's Medium, supplemented with 10% FBS and 2 mM L-glutamine. All media and supplements were obtained from Sigma-Aldrich. Cultures were split one day before transfection (at approximately 0.5 million cells per ml_ before transfection). Cells were transfected with Nucleofector Solution V (Lonza) on a

Nucleofector (Lonza) with the T-016 program. Each nucleofection solution contained approximately 0.6 million cells. Transfection treatments are detailed in Table 7. Cells were grown at 37°C and 5% $CO_2$ immediately after nucleofection.
[0150]

表７．トランスフェクション処理

| 処理 | 修飾されたＣａｓ９ | ガイドＲＮＡ | ドナー配列 |
|---|---|---|---|
| A | アンチリバースキャップアナログを用いて転写されたＣａｓ９ ｍＲＮＡ（１０μｇ）。 | プレアニーリングされたｃｒＲＮＡ－ｔｒａｃｒＲＮＡの二本鎖（０．３ｎｍｏｌ） | ＡＡＶＳ１－ＧＦＰプラスミドＤＮＡ（１０μｇ） |
| B | アンチリバースキャップアナログを用いて転写されたＣａｓ９ ｍＲＮＡ（１０μｇ）。 | キメラＲＮＡ（０．３ｎｍｏｌ） | ＡＡＶＳ１－ＧＦＰプラスミドＤＮＡ（１０μｇ） |
| C | 転写後キャッピング反応によりキャップ構造を付加されたＣａｓ９ ｍＲＮＡ（１０μｇ）。 | キメラＲＮＡ（０．３ｎｍｏｌ） | ＡＡＶＳ１－ＧＦＰプラスミドＤＮＡ（１０μｇ） |
| D | Ｃａｓ９プラスミドＤＮＡ（１０μｇ） | Ｕ６－キメラＲＮＡプラスミドＤＮＡ（５μｇ） | ＡＡＶＳ１－ＧＦＰプラスミドＤＮＡ（１０μｇ） |
| E | なし | なし | ＡＡＶＳ１－ＧＦＰプラスミドＤＮＡ（１０μｇ） |
| F | なし | なし | なし |

表７．トランスフェクション処理　Table 7. Transfection treatments
処理　Treatment
修飾されたＣａｓ９　Modified Cas9
ガイドＲＮＡ　Guide RNA
ドナー配列　Donor sequence
アンチリバースキャップアナログを用いて転写されたＣａｓ９　ｍＲＮＡ（１０μｇ）
Cas9 mRNA transcribed with an Anti-Reverse Cap Analog (10 μg)
プレアニーリングされたｃｒＲＮＡ－ｔｒａｃｒＲＮＡの二本鎖（０．３ｎｍｏｌ）　pre-annealed crRNA-tracrRNA duplex (0.3 nmol)
ＡＡＶＳ１－ＧＦＰプラスミドＤＮＡ（１０μｇ）　AAVS1-GFP plasmid DNA (10 μg)
キメラＲＮＡ（０．３ｎｍｏｌ）　chimeric RNA (0.3 nmol)
転写後キャッピング反応によりキャップ構造を付加されたＣａｓ９ｍＲＮＡ（１０μｇ）　Cas9 mRNA capped via post-transcription capping reaction
Ｃａｓ９プラスミドＤＮＡ（１０μｇ）　Cas9 plasmid DNA (10 μg)
Ｕ６－キメラＲＮＡプラスミドＤＮＡ（５μｇ）　U6-chimeric RNA plasmid

DNA (5 μg)
なし None

[0151]

Fluorescence-activated cell sorting (FACS) was performed 4 days after transfection. FACS data are presented in FIG. 4. The percent GFP detected in each of the four experimental treatment groups (A-D) was greater than in the control treatment groups (E, F), confirming integration of the donor sequence and expression of the fusion protein."

(1-10) "[0152]
Example 5: PCR Confirmation of Targeted Integration

Genomic DNA was extracted from transfected cells with GenElute Mammalian Genomic DNA Miniprep Kit (Sigma) 12 days after transfection. Genomic DNA was then PCR amplified with a forward primer located outside the 5' homologous arm of the AAVS1 -GFP plasmid donor and a reverse primer located at the 5' region of the GFP. The forward primer was 5'- CCACTCTGTGCTGACCACTCT-3' (SEQ ID NO:18) and the reverse primer was 5'- GCGGCACTCGATCTCCA-3' (SEQ ID NO:19). The expected fragment size from the junction PCR was 1388 bp. The amplification was carried out with JumpStart Taq ReadyMix (Sigma), using the following cycling conditions: 98°C for 2 minutes for initial denaturation; 35 cycles of 98°C for 15 seconds, 62°C for 30 seconds, and 72°C for 1 minutes and 30 seconds; and a final extension at 72°C for 5 minutes. PCR products were resolved on 1% agarose gel.
[0153]

Cells transfected with 10 μg of Cas9 mRNA transcribed with an Anti-Reverse Cap Analog (ARCA), 0.3 nmol of pre-annealed crRNA-tracrRNA duplex, and 10 μg of AAVS1 -GFP plasmid DNA displayed a PCR product of the expected size (see lane A, FIG. 5).

(1-11) "
[FIG. 4-1]

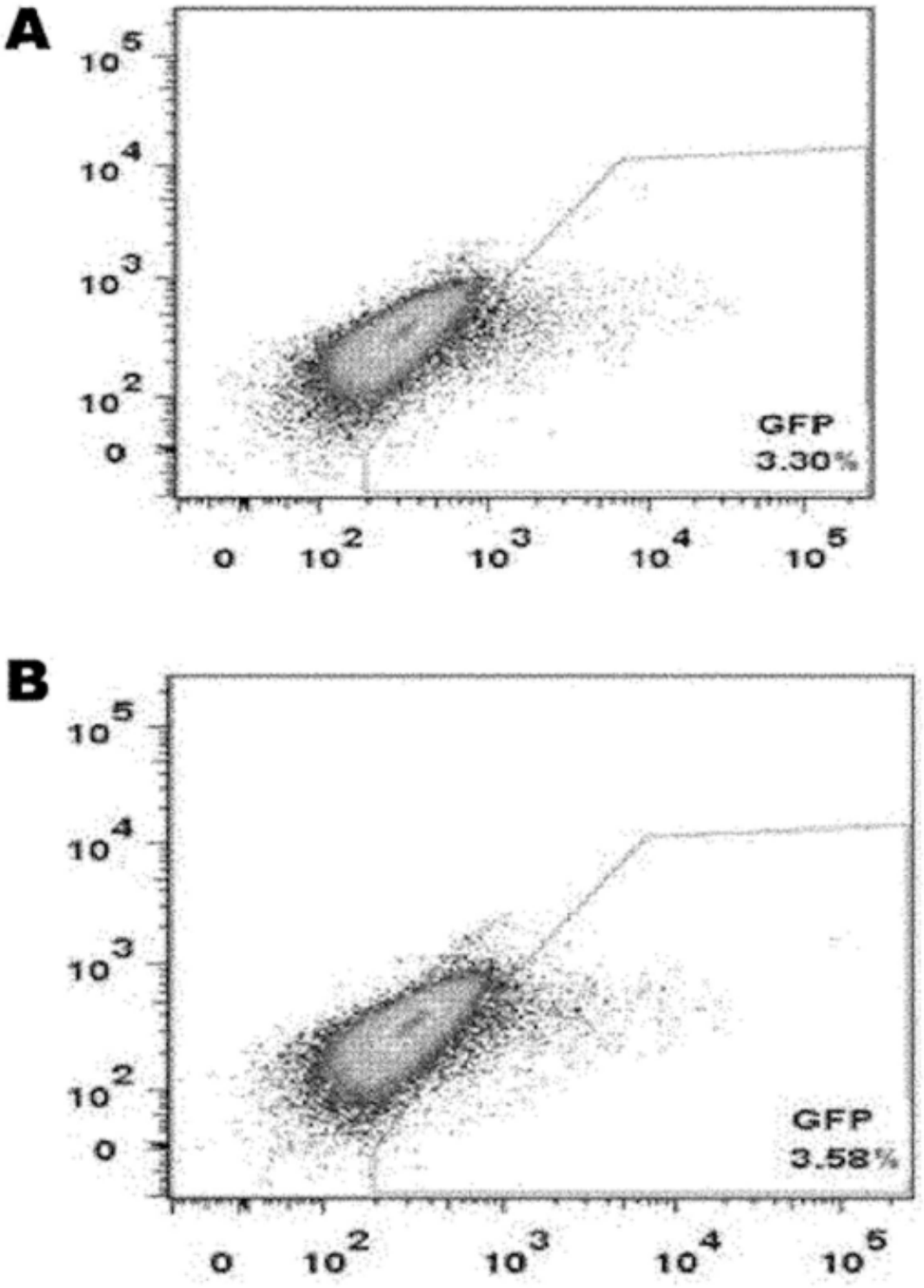

A
$10^5$
$10^4$
$10^3$
$10^2$
0
GFP
3.30%
0
$10^2$
$10^3$
$10^4$
$10^5$
B
$10^5$
$10^4$
$10^3$
$10^2$
0
GFP
3.58%
0
$10^2$
$10^3$
$10^4$
$10^5$

**FIG. 4**

[FIG. 4-2]

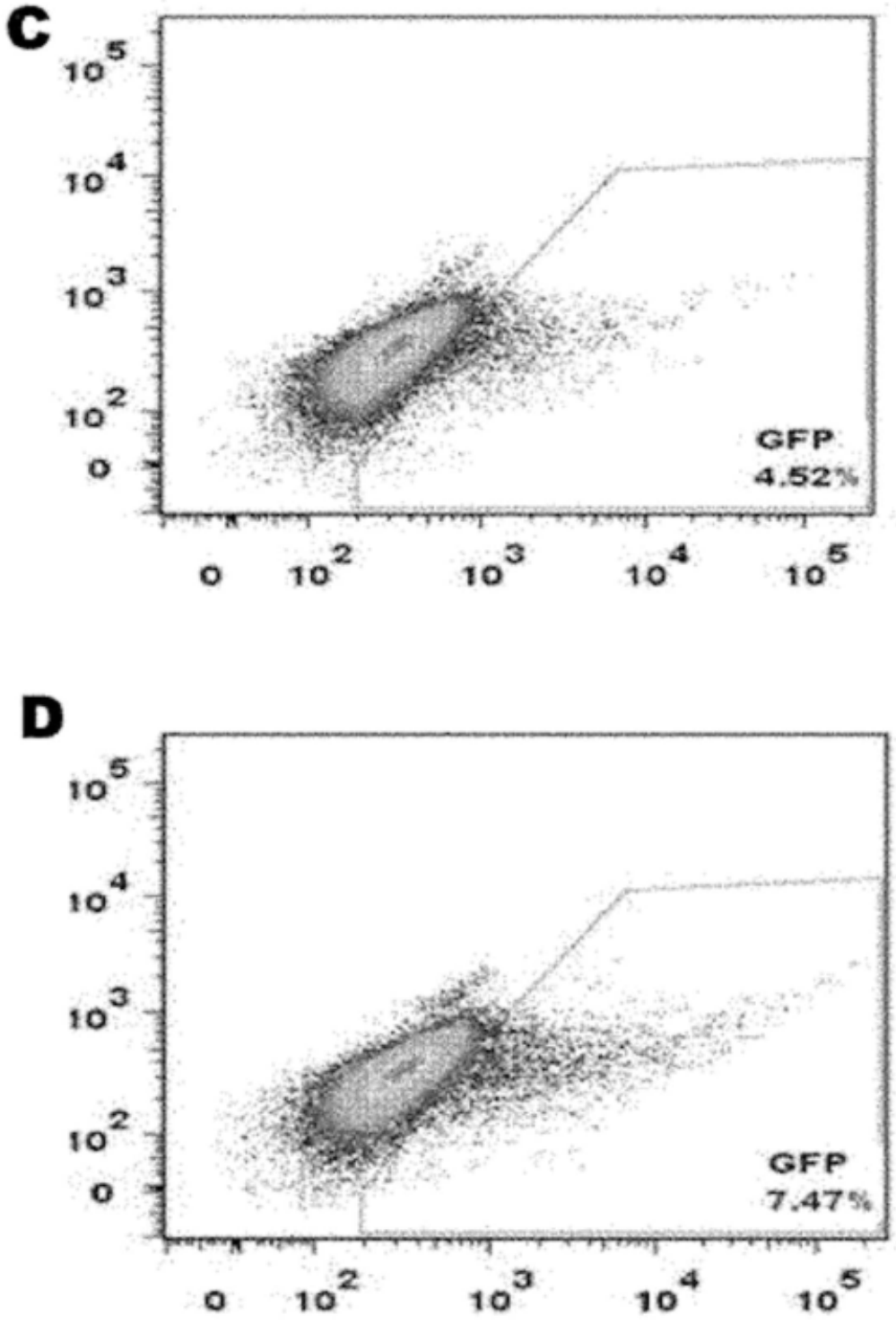

C
$10^5$
$10^4$
$10^3$
$10^2$
0
GFP
4.52%
0
$10^2$
$10^3$
$10^4$
$10^5$
D
$10^5$
$10^4$
$10^3$
$10^2$
0
GFP
7.47%
0
$10^2$
$10^3$
$10^4$
$10^5$

FIG. 4

[FIG. 4-3]

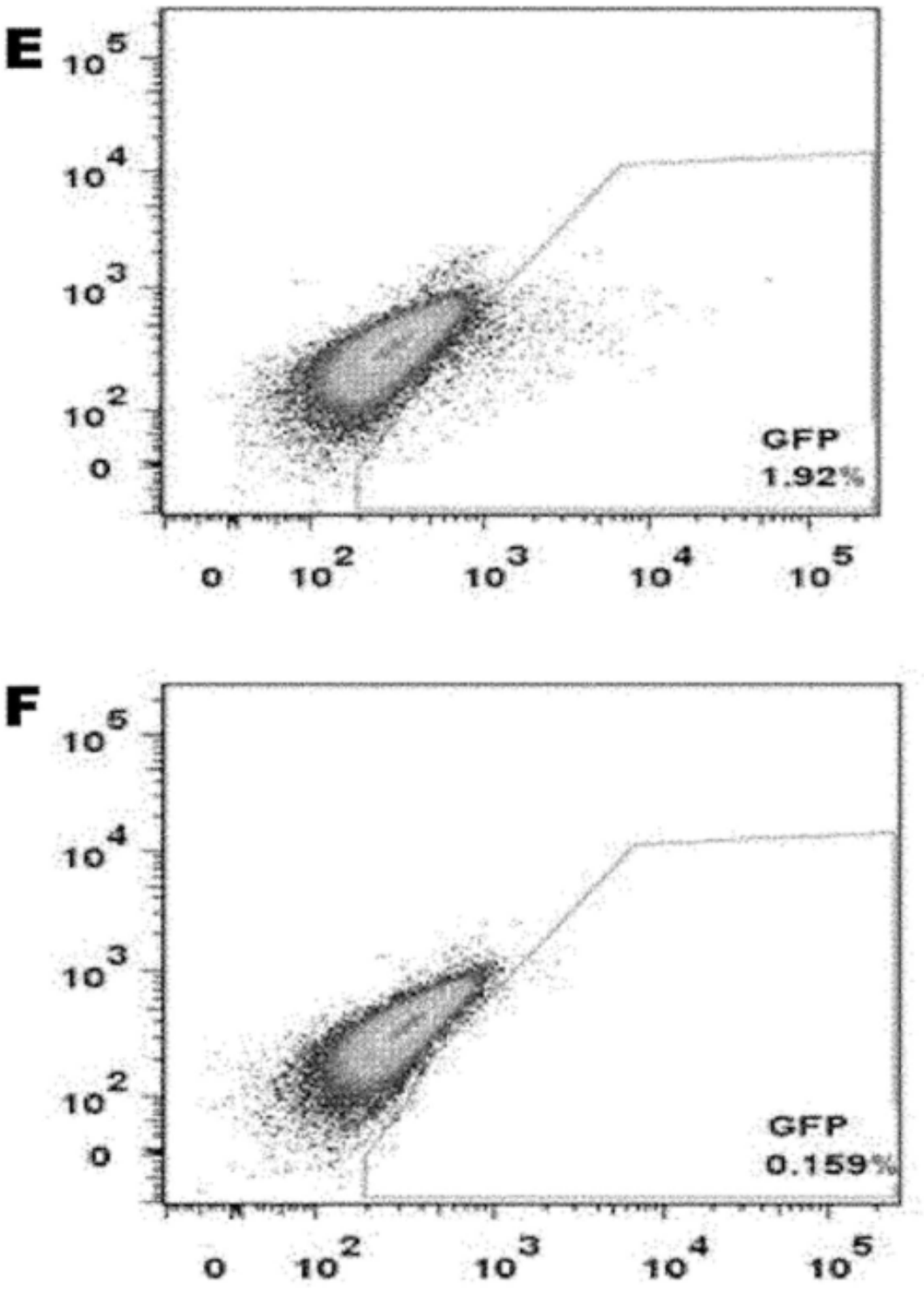

E
$10^5$
$10^4$
$10^3$
$10^2$
0
GFP
1.92%
0
$10^2$
$10^3$
$10^4$
$10^5$
F
$10^5$
$10^4$
$10^3$
$10^2$
0
GFP
0.159%
0
$10^2$
$10^3$
$10^4$
$10^5$

FIG. 4

"

(1-12) "

[FIG. 5]

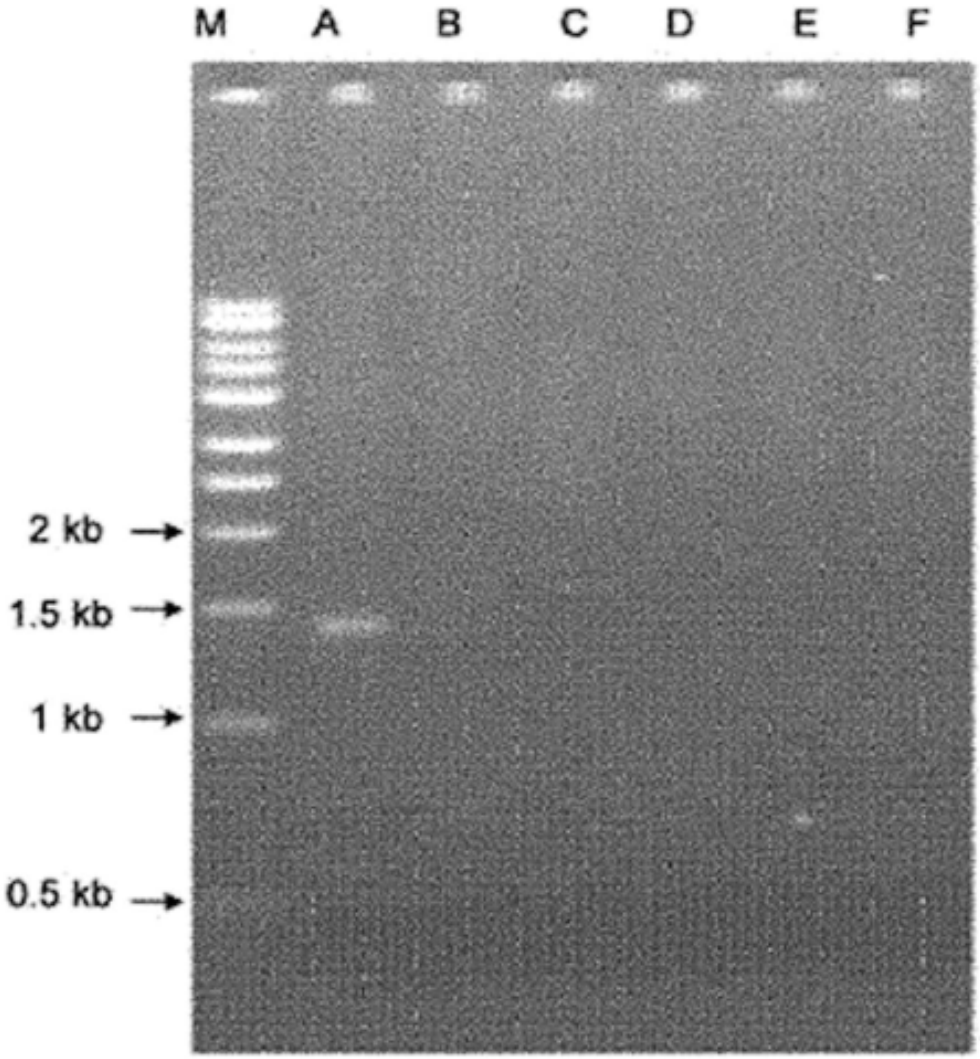



FIG. 5

"

2. Cited Invention

Since not only the spirit of Prior Application 1 is described in the above (1-1) to (1-3), (1-6) and (1-7), but also experimental confirmation is made in the above (1-9) and

(1-11), it is recognized that the originally attached description, etc. of Prior Application 1 describe the following invention (hereinafter, "Cited invention 1").

"A vector system comprising (i) a vector comprising a promoter regulatory sequence operably linked to a nucleic acid encoding at least one Type II Cas9 protein comprising at least one nuclear localization signal, (ii) a vector comprising promoter regulatory sequences operably linked to a DNA encoding at least one guide RNA comprising a first region at the 5' end that is complementary to the target site in the chromosome sequence in the eukaryotic cell, a second internal region that forms a stem-loop structure, and a third 3' region that remains essentially single-stranded, and (iii) a vector comprising at least one donor polynucleotide, wherein the guide RNA guides the Type II Cas9 protein to the target site in the chromosome sequence in the eukaryotic cell and the Type II Cas9 protein induces chromosomal DNA double-strand break at the target site and the double-strand break is repaired by a DNA repair process so that the chromosome sequence is modified."

3. Comparison and Judgment
(1) Component (a) of the Invention
i. "Guide sequence"

With respect to "guide sequence" of the Invention, while [0073] in the description of the present application describes that "in general, the guide sequence is an optional polynucleotide sequence that has sufficient complementation with the target polynucleotide sequence for hybridizing with the target sequence and directing sequence-specific binding of the CRISPR complex to the target sequence," judging from the description in the above (1-4), and (1-6), since "a first region at the 5' end that is complementary to the target site in the chromosome sequence in the eukaryotic cell" of Cited Invention 1 forms a guide RNA together with the second internal region that forms a stem loop structure, and the third 3' region that remains essentially single-stranded, and the guide RNA guides Cas9 to the target site in the specific chromosome sequence by forming a complex with the Cas9 protein, it corresponds to "guide sequence" of the Invention.

ii. "tracrRNA and tracr mate sequence"

With respect to "tracrRNA and tracr mate sequence" of the Invention, it is described that "the CRISPR complex comprises CRISPR enzyme that forms a complex with a guide sequence that is hybridized with the target sequence in the polynucleotide, and, then, the guide sequence binds to the tracr mate sequence that is hybridized with tracr sequence" in [0017] in the description of the present application, that "in certain embodiments, the CRISPR enzyme is a Cas9 enzyme" in [0015], and that "in certain embodiments, tracr sequence and tract mate sequence (note by the body: It is recognized as erroneous description of "tracr mate sequence," hereinafter, the same applies to any cited portion) are contained in a single transcript, and, as a result, generate a hybridization secondary structure between the two, for example, a transcript that has a hairpin. A preferable loop-forming sequence used in the hairpin structure is 4 nucleotides in length, and, most preferably, has a sequence, GAAA" in [0076].

Judging from those descriptions, it can be said that "tracrRNA" and "tracr mate sequence" of the Invention hybridized with each other, and, together with "guide

sequence" and "Cas9," form "CRISPR complex," and, if "tracrRNA" and "tracr mate sequence" are contained in the single transcript, form a hairpin structure by hybridizing with each other.

On the other hand, judging from the descriptions in the above (1-4) and (1-6), it can be said that "a second internal region that forms a stem-loop structure, and a third 3' region that remains essentially single-stranded" in Cited Invention 1 form together with the "first region at the 5' end that is complementary to the target site in the chromosome sequence in the eukaryotic cell e" (corresponds to "guide sequence" of the Invention as stated in the above i), a complex with "Cas9 protein," and the "stem-loop structure" contained in the "second internal region" corresponds to the hairpin structure formed when the "tracrRNA" and the "tracr mate sequence" in the Invention are contained in the single transcript through the loop-forming sequence.

Accordingly, "a second internal region that forms a stem-loop structure, and a third 3' region that remains essentially single-stranded" of Cited Invention 1 correspond to a sequence containing "tracrRNA and tracr mate sequence" of the Invention; namely, a sequence composed of the "tracrRNA," "tracr mate sequence," and a loop-forming sequence.

iii. "Regulatory element"

With respect to the " regulatory element" of the Invention, since it is described in [0010] of the description of the present application that "the term 'regulatory element' covers promoters, enhancers, internal ribosome entry site (IRES), and other expression control elements (for example, transcription termination signal, for example polyadenylation signal and poly U sequence)," the "promoter regulatory sequence" of Cited Invention 1 corresponds to "regulatory element" of the Invention.

iv. Since it is just as explained in the above items i to iii, it can be said that (ii) in Cited Invention 1 is a vector that comprises component (a) of the Invention.

(2) Component (b) of the Invention

In the light of the description in [0071] of the specification of the present application, "one or more Nuclear Localization Signals (NLS)," and the common general knowledge that NLS is an abbreviation of Nuclear Localization Signal, since it is obvious that "(NLS (including plural form))" in the Invention is a synonym of "nuclear localization signal (including plural form)" added written in the brackets, item (i) of Cited Invention 1 is a vector comprising the component (b) of the Invention, and satisfies "further comprises one or more Nuclear Localization Signals (NLS (including plural form)) expressed together with the nucleotide sequence encoding the Cas9 protein" in the Invention.

(3) Component (c) of the Invention

With respect to "recombinant template" of the Invention, [0077] of the description of the present application describes that "in some embodiments, the recombinant template is, for example, designed to function as a template in homologous recombination in the target sequence, which is nicked or cleaved by the CRISPR enzyme as a part of the CRISPR complex, or its neighborhood."

It can be said from the description in the above (1-5) that the "donor

polypeptide" in Cited Invention 1 can be substituted with a chromosome sequence or inserted into a chromosome sequence at the target site during repairing mediated by the Homologous recombination repair process.

Accordingly, Cited Invention 1, (iii) corresponds to a vector comprising the component (c) of the Invention.

(4) While, in the Invention, "the above guide sequence targets the one or more polynucleotide loci in the eukaryotic cells and the Cas9 protein cleaves the one or more polynucleotide loci whereby sequence of the one or more polynucleotide loci is modified," since, in Cited Invention 1 also, "the guide RNA guides the Type II Cas9 protein to the target site in the chromosome sequence in the eukaryotic cell, and the Type II Cas9 protein induces at the target site the chromosome DNA double-strand break, and the double-strand break is repaired by DNA repair process so that the chromosome sequence is modified," they also do not differ from each other in this point.

(5) While the Invention relates to "An engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR-associated (Cas) (CRISPR-Cas) vector system", since Cited Invention 1 relates to a vector system that has a vector obtained by modifying naturally existing Type II Cas9 protein as a constituent component as it comprises "at least one nuclear localization signal", it corresponds to the "engineered, non-naturally occurring" vector system of the Invention.

On the other hand, while it is obvious that "CRISPR" is an abbreviation of "Clustered Regularly Interspaced Short Palindromic Repeat," and "Cas" is an abbreviation of "CRISPR-associated," and, "(CRISPR/Cas)" of the Invention is just a synonym of "Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-CRISPR-associated (Cas)" added written in brackets, since "Type II Cas9 protein" in Cited Invention 1 is derived from "Type II CRISPR/Cas system" as explained in the above (1-3), Cited Invention 1 is a "Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-CRISPR-associated (Cas) CRISPR-Cas vector system," and they do not differ from each other also in this regard.

(6) Summary

As explained above, the Invention is the same as Cited Invention 1.

4. Appellant's allegation

Appellant's allegations in the written demand for trial after corrections by the written amendment dated November 1, 2017 and judgments by the collegial body on Appellant's allegations are shown below.

(1) Appellant's allegation

Among Examples in the originally attached description, etc. of Prior Application 1, all components are DNA only in the treatment D in Example 4, and it can be deemed as a CRISPR/Cas vector system, but, since it is not disclosed that polynucleotide locus as the target in the eukaryotic cell is cleaved and modified, the Invention is not identical or substantially identical to the invention described in Prior Application 1.

Namely, the examiner found cleavage and modification of the target polynucleotide with increase in the GFP signal in FIG. 4D in the above (1-11) that is the

result of the treatment D in Example 4, but the increase is very small, and it cannot be concluded that the cleavage or modification actually occurred. Rather, in the experiment in Example 5 that was for verifying incorporation of donor sequence into the target polynucleotide, incorporation was confirmed in the treatment A in Example 4 (not any vector system), (lane A in FIG. 5 of the above (1-12)), but the result was negative with respect to treatment D (lane D)). Accordingly, a person skilled in the art who referred to Examples 4 and 5 together must have concluded that the originally attached description, etc. of Prior Application 1 does not disclose any CRISPR/Cas vector system that can cleave or modify the target polynucleotide.

(2) Judgment

i. Example 4 in Prior Application 1

(i) Donor polynucleotide of Example 4

In Table 7 of the above (1-9), Example 4 describes that "AAVS1-GFP plasmid DNA" was used as the donor polynucleotide.

Then, from the description in the above (1-8), it can be understood that the donor polynucleotide has a structure of the homologous arm of AAVS1 locus of 5' (1185bp), RNA splicing receptor, turbo GFP coding sequence, 3' transcript terminator and homologous arm of AAVS1 locus of 3' (1217bp), and RNA splicing between the first exon of PPP1R12C and the splice receptor occur by specific gene transfer in AAVS1 locus that is the target site in the chromosome sequence (it corresponds to "incorporation of the donor sequence to the target polynucleotide" in the above Appellant's allegation.), and, as a result, GFP is expressed by generation of the fusion protein from first 107 amino acids of PPP1R12C and turbo GFP. Namely, it can be said that GFP is not expressed only if the donor polynucleotide exists in cells or nucleus, and GFP is expressed only after incorporation of the donor sequence into the target polynucleotide in the chromosome sequence in the eukaryotic cell has occurred.

(ii) Result of Example 4

"Cas9 plasmid DNA", "U-6 chimeric RNA plasmid DNA" and "AAVS1-GFP plasmid DNA" described as "treatment D" in Table 7 of the above (1-9) correspond to "(i) a vector comprising promoter regulatory sequences operably linked to a nucleic acid encoding at least one Type II Cas9 protein comprising at least one nuclear localization signal", "(ii) a vector comprising promoter regulatory sequences operably linked to a DNA encoding at least one guide RNA comprising a first region at the 5' end that is complementary to the target site in the chromosome sequence in the eukaryotic cell, a second internal region that forms a stem-loop structure, and a third 3' region that remains essentially single-stranded" and "(iii) a vector comprising at least one donor polynucleotide", respectively, in Cited Invention 1. With respect to the results of the experiments using it, the originally attached description, etc. of Prior Application 1 describes that "the rate of GFP detected in each of four experimental treatment groups (A to D) was larger than that of control treatment groups (E and F), and insertion of donor sequences and expression of fusion protein were confirmed" (above (1-9)).

For confirmation, examining the experimental data in detail, the above vector was transfected to human K562 cells, and FACS data that indicate the incidence rate of GFP carried out four days later are shown as FIG. 4D, and the value was "7.47%." Since "treatment F" of Table 7 does not transfect any vector, "0.159%" indicated in FIG.

4F can be deemed as the background value of this experimental system. Accordingly, it can be said that "7.311%" obtained by deducting "0.159%" from "7.47%" is the result brought by carrying out the treatment D.

On the other hand, since only "AAVS1-GFP plasmid DNA" is used in the "treatment E" in Table 7, it can be said that the result reflects incorporation of donor sequence into the target polynucleotide not depending on "Cas9 plasmid DNA" and "U-6 chimeric RNA plasmid DNA." Then, "1.761%" obtained by deducting the background value, "0.159%" from "1.92%" shown in FIG. 4E is the result obtained by carrying out the treatment E.

Comparing the results of the "treatment D" and the "treatment E," a value not less than four times the value obtained in the "treatment E" was obtained in the "treatment D," and since it can be said that the difference was brought by using "Cas9 plasmid DNA" and "U-6 chimeric RNA plasmid DNA," it is reasonable to understand that, in the "treatment D," cleavage of the target polynucleotide and incorporation of the donor sequence were promoted by the "Cas9 plasmid DNA" and the "U-6 chimeric RNA plasmid DNA." This is exactly as described in the above (1-9).

As explained above, it is obvious that, in the light of the method and result of the experiment in Example 4, Appellant's allegation concerning Example 4 is not justifiable.

ii. Example 5 of Prior Application 1

In Example 5 of the above (1-10), PCR was carried out for confirming the incorporation of the donor sequence into the target polynucleotide, and the result is shown in FIG. 5. It cannot be confirmed from FIG. 5 that, if the above "treatment D" is carried out, a PCR product which shows that the incorporation was conducted is obtained.

Since the results of Examples 4 and 5 were obtained by using different samples, however, while genomic DNA was derived from cells 12 days after the transfection in Example 5, analysis was made 4 days after transfection in Example 4, the result of Example 4 cannot be directly denied from the result of Example 5.

iii. Descriptions in the originally attached description, etc. of Prior Application 1 as a whole

As stated in "2 Cited Invention" above, the originally attached description, etc. of Prior Application 1 discloses Cited Invention 1 and, as stated in the above item i, Example 4 indicates in an embodiment that cleavage of the target polynucleotide and incorporation of the donor sequence occur.

Accordingly, even if the result of Example 5 contains any point inconsistent with the above, it cannot be said that because of such inconsistency, Cited Invention 1 is not disclosed in the originally attached description, etc. of Prior Application 1.

iv. As explained in the above items i to iii, Appellant's allegations cannot be accepted.

5. Summary

As explained above, since the Invention is the same as Cited Invention 1, and, in addition, the inventor in this application is not the same as the person who invented the foreign language patent application before the filing of the present application, and, at the time of the filing of the present application, the applicant is not the same as the

applicant of the above foreign language patent application, Appellant should not be granted a patent under the provisions of Article 29-2 of the Patent Act (see Article 184-13 of the Patent Act).

No. 5 Reason 2 (Article 29(2) of the Patent Act)

1. Description in Cited Document
(1) Cited Document 2

Cited Document 2 (Science, Aug 2012, Vol. 337, pp. 816-821, Supplementary Materials) cited in the examiner's decision that is a publication distributed before the first priority date of the present application describes an academic article titled "A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity" that describes the following matter.

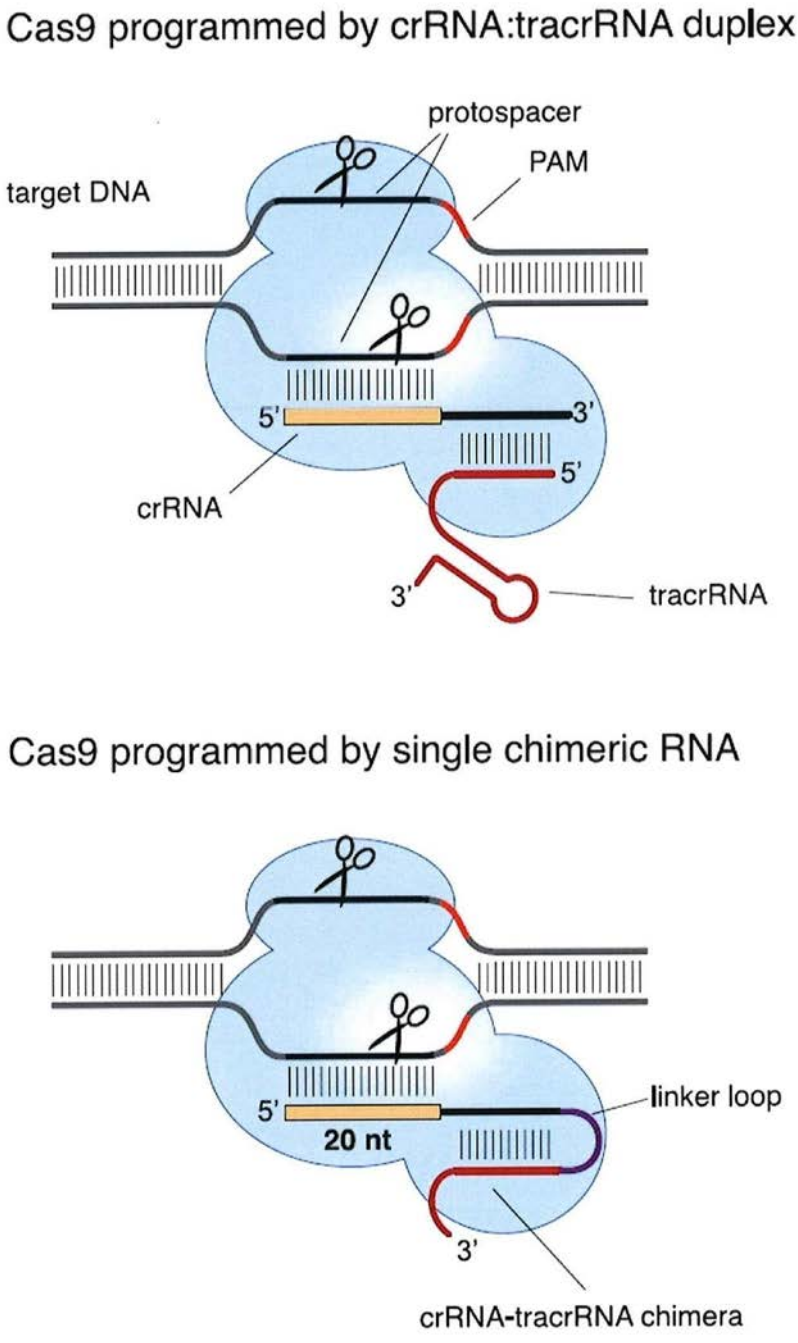
(1-1) "Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. ... The dual-tracrRNA: crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing." (Abstract)

(1-2) "We show here that in type II systems, Cas9 proteins constitute a family of enzymes that require a base-paired structure formed between the activating tracrRNA and the targeting crRNA to cleave target dsDNA. Site-specific cleavage occurs at locations determined by both base-pairing complementarity between the crRNA and the target protospacer DNA and a short motif [referred to as the protospacer adjacent motif (PAM)] juxtaposed to the complementary region in the target DNA" (page 816, middle column, lines 25 to 35).

(1-3) "We designed two versions of a chimeric RNA containing a target recognition sequence at the 5' end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA ... In cleavage assays using plasmid DNA, we observed that the longer chimeric RNA was able to guide Cas9-catalyzed DNA cleavage in a manner similar to that observed for the truncated tracrRNA:crRNA duplex." (page 820, left column, lines 5 to 18).

(1-4) "FIG. 5. Cas9 can be programmed using a single engineered RNA molecule combining tracrRNA and crRNA features. (A) (Top) In type II CRISPR/Cas systems, Cas9 is guided by a two-RNA structure formed by activating tracrRNA and targeting crRNA to cleave site-specifically-targeted dsDNA. (Bottom) A chimeric RNA generated by fusing the 3' end of crRNA to the 5' end of tracrRNA.

A




"

(1-5) "Zinc-finger nucleases and transcription-activator-like effector nucleases have attracted considerable interest as artificial enzymes engineered to manipulate genomes. We propose an alternative methodology based on RNA-programmed Cas9 that could offer considerable potential for gene-targeting and genome-editing applications" (page 820, right column, lines 2 to 9).

(1-6) "Plasmid DNA cleavage assay.
...
Native or restriction digest-linearized plasmid DNA (300 ng (~8 nM)) was incubated for 60 min at 37°C with purified Cas9 protein (50-500 nM) and tracrRNA:crRNA duplex (50-500 nM, 1:1) in a Cas9 plasmid cleavage buffer (20 mM HEPES pH 7.5, 150 mM KCL, 0.5 mM DTT, 0.1 mM EDTA) with or without 10 mM MgCl2" (SUPPLEMENTARY MATERIALS AND METHODS).

(2) Well-known example 1

Similarly, Gene Therapy, 2008, Vol. 15, pp. 1463-1468 that is a publication distributed before the first priority date of the present application is a review article on one of genome editing technologies, zinc-finger nuclease (ZFN), and describes the following matters.

(2-1) "A ZFN-induced break can be used for gene editing in two different modes. ... To achieve the type of sequence replacement that is usually considered gene targeting, a donor DNA that is largely homologous to the target, but carries the specific desired sequence change, is delivered along with the ZFNs. When HR uses this donor as a template, the change is incorporated at the target" (page 1463, right column, last paragraph).

(2-2) "Published reports have described successful ZFN-induced targeting of 14 genes in various different organisms at their endogenous chromosomal locations: 6 in mammalian cells, 3 in zebrafish, 3 in *Drosophila*, and 1 each in nematodes and in plant cells" (page 1464, right column, lines 1 to 5).

(2-3)
"They also produced a combination vector having one ZFN and 1 copy of donor DNA. By this, the number of distinguished viruses needed for infecting the same cell decreases." (page 1465, left column, lines 29 to 32).

(2-4) "In this case, both ZFNs were delivered in a single, bicistronic vector, with their expression driven by a CMV promoter" (page 1465, left column, lines 42 to 44).

(3) Well-known example 2

Similarly, Methods, 2011, Vol. 53, pp. 339-346 that is a publication distributed before the first priority date of the present application describes the following matters.

(3-1) "higher efficiencies are achieved using technology based on zinc-finger nucleases (ZFNs). ZFNs are molecular scissors that induce DNA double-strand breaks (DSBs) at prespecified genomic sites. ... The enzymatically induced DSB can be repaired either by error-prone non homologous end-joining (NHEJ), leadind to short insertions or deletions or homology-directed repair (HDR), based on homologous recombination between an exogenous donor DNA and the target locus. ... Typically, a ZFN-induced DSB increases the frequency of homologous recombination several thousand-fold. Overall, ZFN technology has been successfully applied in more than ten

organisms, including drosophila, plants, zebra fish, rat, and mouse or human pluripotent stem cells" (page 339, right column, the last paragraph to page 340, left column, line 3 from the bottom).

(4) Well-known example 3

Similarly, Nature Biotechnology, 2011, Vol. 29, pp.143-148 that is a publication distributed before the first priority date of the present application described the following matters.

(4-1) "These results have spurred interest in the potential use of TALE-nuclease chimeras (TALENs) as site-specific endonuclease for selective genome cleavage. Here we report the development of TALENS capable of mediating efficient endogenous gene modification. First, we demonstrate TALE activity in a mammalian cell environment through targeted regulation of episomal reporters and an endogenous gene" (page 143, right column, lines 8 to 13).

(4-2) "Next, these TALENs were introduced into K562 cells with a donor DNA fragment designed to transfer a 46-bp insert encoding a BgII restriction site into the targeted locus. PCR amplification, followed by BgII digestion revealed efficient edition, with up to 16% of alleles possessing the inserted sequence (FIG. 5). This result shows that the TALEN architecture described here can induce precise gene editing by HDR" (page 146, right column, last line to page 147, left column, line 5).

(5) Well-known example 4

Similarly, Nucleic Acids Research, 2011, Vol. 39, e82 that is a publication distributed before the first priority date of the present application described the following matters. Since it is written in English language, the translation prepared by the body is shown below.

(5-1) "As a result, TAL effectors have attracted great interest as DNA targeting tools. In particular, we and other groups have shown that TAL effectors can be fused to the catalytic region of the FokI nuclease to create DNA double-strand breaks (DSBs) *in vivo* for genome editing ... DSBs are repaired in nearly all cells by one of two highly conserved processes, non-homologous end joining (NHEJ), which often result in small insertions or deletions and can be harnessed for gene disruption, and homologous recombination (HR), which can be used for gene insertion or replacement" (page 2, left column, lines 5 to 19).

(5-2) "We show that TALENs targeted with this software and constructed using the plasmid set are active in a yeast DNA cleavage assay and effective in gene targeting in human cells and Arabidopsis thaliana (hereafter Arabidopsis*)* protoplasts" (page 2, right column, lines 35 to 40).

(5-3) "One of the pairs of TALENs targeting the human HPRT1 gene was subcloned into the mammalian expression vector pCDNA3.1(-) (Invitrogen) using XhoI and AflII. These enzymes excise the entire TALEN from pTAL3 or pTAL4 and place the coding sequence under control of the CMV (cytomegalovirus) promoter. The resulting

plasmids were introduced into HEK293T cells by transfection using Lipofectamine 2000 (Invitrogen) following the manufacturer's protocol" (page 6, left column, lines 14 to 6 from the bottom).

(6) Well-known example 5

Similarly, international publication No. 2012/012738 that is a publication distributed before the first priority date of the present application described the following matters. Since it is written in English language, described matters of and the source of citation in National publication of International Patent Application No. 2013-537410 that is the national publication of the above publication is shown as a translation.

(6-1) "[Claim 1]

A method for editing at least one endogenous chromosomal sequence in a cell, the method comprising:

a) introducing into the cell (i) at least one targeting endonuclease or nucleic acid encoding a targeting endonuclease, the targeting endonuclease being able to introduce a double-stranded break at a targeted cleavage site in the chromosomal sequence, and (ii) at least one single-stranded nucleic acid comprising a first portion having substantial sequence identity to the chromosomal sequence on at least one side of the targeted cleavage site; and

b) maintaining the cell under conditions such that the double-stranded break introduced by the targeting endonuclease is repaired by a homology-directed process such that the chromosomal sequence is exchanged with the sequence of the single-stranded nucleic acid, thereby editing the chromosomal sequence. ...

[Claim 13]

The method of claim 1, wherein the targeting endonuclease is a zinc finger nuclease, a meganuclease, transcription activator-like effector (TALE), nuclease, a site-specific nuclease, or an artificial targeted DNA double strand break inducing agent.

(6-2) "[0051]

... In general, the nucleic acid encoding the targeting endonuclease will be operably linked to a promoter control region."

(6-3) "[0064]

(d) Cells

The method comprises introducing into a cell the targeting endonuclease molecules(s) and nucleic acid(s) described above. A variety of cells are suitable for use in the method. In general, the cell will be a eukaryotic cell or a single cell eukaryotic organism."

(7) Well-known example 6

Similarly, National publication of International Patent Application No. 2001-503971 that is a publication distributed before the first priority date of the present application describes the following matters.

(7-1) "the marked gene consists of Escherichia coli β-galactosidase reporter gene, and its expression can be easily detected by staining with X-Gal (4-chloro-5-bromo-3-

indolyl-β-D-galactopyranoside). This gives a sequence that encodes its 3' part with a eukaryotic cell nuclear localization signal. By nuclear localization of the recombinant β-galactosidase, it became possible to exclude the problem of background noise caused by the cross-reaction with endogenous β-galactosidase of the host cell that can also be detected by Xgal, and, therefore, specific detection of enzymic activity from fixed plasmid becomes possible" (page 23, line 2 from the bottom to page 24, line 6).

(8) Well-known example 7

Similarly, Japanese Unexamined Patent Application Publication No. Hei 10-80274 that is a publication distributed before the first priority date of the present application describes the following matters.

(8-1) "[Claim 1] A recombinant gene comprising a nuclear transport signal (NLS) gene and a tetracycline transactivator (tTA) gene."

(9) Well-known example 8

Similarly, National publication of International Patent Application No. 2002-538842 that is a publication distributed before the first priority date of the present application describes the following matters.

(9-1) "[0147]

(Introduction of nuclear localization signal promotes tetR-mediated repression) As tetR distribution was observed mainly in cytoplasm, nuclear localization signal (NLS) was introduced to 3' end of tetR gene and introduction into nucleus was promoted, and repression of tetR-mediated transcription was enhanced as a result."

2. Cited Invention

From the above (1-1) to (1-4), and (1-6) (particularly, FIG. 5A, lower diagram) (hereinafter, (1-1), etc., in No. 5 means summary in (1-1), etc. in No. 5, 1), it has been experimentally confirmed in Cited Document 2 by a CRISPR/Cas system, in which chimeric RNA designed based on tracrRNA and crRNA that compose the CRISPR/Cas system and Type II Cas9 protein are used, that the target sequence is cleaved in buffer solution. Accordingly, since it can be said that Cited Document 2 describes an engineered CRISPR/Cas system that does not occur naturally, it is recognized that Cited Document 2 describes the following invention (hereinafter, "Cited Invention 2").

"An engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR-associated (Cas) (CRISPR/Cas) system comprising
a) a chimeric RNA that comprises a target recognition sequence at the 5' end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA, and in which the target recognition sequence hybridizes with the target sequence in buffer solution, and
b) Type II Cas9 protein,
whereby the Cas9 protein cleaves the target sequence.

3. Comparison

With respect to "guide sequence" of the Invention, it is described in [0073] in the description in the present application that "generally, the guide sequence is an optional polynucleotide sequence that has sufficient complementation with the target polynucleotide sequence for hybridizing with the target sequence and directing sequence-specific binding of the CRISPR complex to the target sequence."

In addition, as shown in the above No. 4, 3, (1), ii, "tracrRNA" and "tracr mate sequence" of the Invention hybridize with each other and form a "CRISPR complex" together with "guide sequence" and "Cas9" and, if a "tracrRNA" and a "tracr mate sequence" are contained in any single transcript, it can be said that they form a hairpin structure by hybridizing with each other.

Meanwhile, the chimeric RNA of Cited Invention 2 also comprises "a target recognition sequence at the 5' end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA," and forms a complex with a Cas9 protein, and the "target recognition sequence" "hybridizes with the target sequence" and "thereby ... cleaves the target sequence."

Accordingly, "a) a chimeric RNA that comprises a target recognition sequence at the 5' end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA," and in which "the target recognition sequence" "hybridizes with the target sequence" in Cited Invention 2 is "a) CRISPR/Cas system polynucleotide comprising guide sequence, tracrRNA and tracr mate sequence" of the Invention, and "the guide sequence" corresponds to CRISPR/Cas system polynucleotide that "hybridizes with the target sequence."

Judging from the above, corresponding features and different features between the Invention and the Cited Invention 2 are as shown below.

[Corresponding features]

"An engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR-associated (Cas) (CRISPR/Cas) system comprising
a) a CRISPR/Cas system polynucleotide comprising a guide sequence, a tracrRNA and a tracr mate sequence, wherein the guide sequence hybridizes to a target sequence and
b) Type II Cas9 protein,
whereby the Cas9 protein cleaves the target sequence."

[Different Feature 1]

While in the Invention the guide sequence "hybridizes with one or more target sequences in polynucleotide loci in the eukaryotic cells," and the guide sequence targets "the one or more polynucleotide loci in the eukaryotic cells" and Cas9 protein cleaves it by further comprising "one or more nuclear localization signals (NLSs) expressed with the nucleotide sequence that encodes the Cas9 protein, in Cited Invention 2 the target sequence exists in buffer solution and Cas9 protein does not have any nuclear localization signal.

[Different Feature 2]

While "recombinant template" is comprised and "the one or more sequences of polynucleotide loci are modified" in the Invention, in Cited Invention 2 no recombinant

template is comprised, and only the target sequence is cleaved.

[Different Feature 3]

While the Invention relates to a CRISPR/Cas "vector" system comprising one or more vectors comprising "the first regulatory element operably linked to nucleotide sequences that encode" CRISPR/Cas system polynucleotide sequence and "the second regulatory element operably linked to a nucleotide sequence that encodes" Type II Cas9 protein, "wherein components (a), (b) and (c) are located on a vector of the same or different system," Cited Invention 2 relates to a CRISPR/Cas system in which a "chimeric RNA" and a "Cas9 protein" are used.

4. Judgment

(1) Regarding Different Feature 1

As explained in the above (1-1) and (1-5), Cited Document 2 describes also that, in addition to the fact that CRISPR/Cas system might deliver a great potential ability toward application in gene targeting and genome editing, the technique based on CRISPR/Cas system could be an alternative to zinc-finger nuclease that is an artificial enzyme designed for genome engineering and transcriptional activation-like effector nuclease.

As explained in the above (2-2), (3-1), (4-1), (5-2), and (6-3), as of the first priority date of the present application, since it was well known that the primary object of genome editing was eukaryotic cells, and zinc-finger nuclease and transcriptional activation-like effector nuclease cleave the target DNA in eukaryotic cells, in other words genome in the nucleus, it is a quite normal idea for a person skilled in the art to try to make the CRISPR/Cas system of Cited Invention 2 function to act on genomes in eukaryotic cells.

In addition, in realizing it, it was a matter that a person skilled in the art could do without any special inventiveness to add a nuclear localization signal to a Cas9 protein as a common practice to transfer a protein into a nucleus (see above (7-1), (8-1), and (9-1)) in order to make the CRISPR/Cas system reach the genome in the nucleus of the eukaryotic cell.

(2) Regarding Different Feature 2

Genome editing means to modify the target gene and rewrite the genome information, and, since it is just a common technique, following DNA breakage with nuclease, to carry out homologous recombination by a recombinant template (see above (2-1), (3-1), (4-2), and (5-1)), as far as Cited Document 2 suggests application of CRISPR/Cas system of Cited Invention 2 to genome editing as explained in above (1-1) and (1-5), it is obvious that concomitant use with a recombinant template is not a matter that requires any inventive faculty to a person skilled in the art.

(3) Regarding Different Feature 3

In trying to make desired protein or nucleic acid work inside cells, it is a usual practice to use a vector that expresses them. As stated in the above (1) and (2), as far as there is sufficient motivation to make the CRISPR/Cas system of Cited Invention 2 work on the genomes in eukaryotic cells, it is a matter that a person skilled in the art could appropriately do to use vector systems for components that compose the system;

namely Cas9 protein, chimeric RNA, and recombinant template. In fact, before the priority date of the Invention, it was common to introduce components for genome editing as vector systems into cells (above (2-3), (2-4), (5-3), and (6-2)).

(4) Summary

As described above, a person skilled in the art could have easily made the Invention based on the invention described in Cited Document 2 and well-known arts before the priority date for the Invention.

5. Appellant's allegation

Appellant's allegation made in the written amendment dated November 1, 2017 after the correction and the judgment by the collegial body to Appellant's allegation are as shown below.

(1) Appellant's allegation

i. Disclosure of Cited Document 2

Cited Document 2 discloses only simple DNA cleavage in vitro and does not contain any information on whether a CRISPR/Cas protein-guide RNA complex can be formed or maintained in eukaryotic cells.

Cited Document 2 neither discloses nor suggests any element important for use in eukaryotic cells. For example, Cited Document 2 neither discloses nor suggests a CRISPR/Cas system comprising nuclear localization signal (NLS), a regulatory element concerning expression of CRISPR/Cas system polynucleotide or Cas9 protein in eukaryotic cells, and a vector system comprising the regulatory element and CRISPR/Cas system nucleotide sequence.

ii. Reasonable expectation of success

Due to the reasons that a eukaryotic cell and a prokaryotic cell differ from each other in the gene expression mechanism, protein folding, existence of RNAi pathway, cytotoxicity by RNA, cell compartmentation, chromatin structure, $Mg^{2+}$ concentration, ribonuclease, etc., and that a targetron system comprising an RNA component and a protein component could not be adapted to routinely function in eukaryotic cells even 16 years after the study, etc., it was not possible to have reasonable expectation that CRISPR/Cas9 could be successfully used in eukaryotic cells.

iii. Contemporary evidences

The following contemporary evidences show that a person skilled in the art did not expect any success. On November 1, 2017, Appellant corrected the written demand for trial and, at the same time, submitted Reference Materials 1 to 15 as a supplemental statement, but it is not clearly shown on what Reference Material each "contemporary evidence" is based.

(iii-1) Dr. Dana Carroll

"What is the activity of this system in eukaryotic cells? Both zinc finger and TALE module derive from natural transcription factors that bind those targets in the context of chromatin. This does not apply to CRISPR component. There is no guarantee if Cas9 efficiently functions on chromatin target, or if required DNA-RNA hybrid could be stabilized under the situation. This structure can be the ground

substance for RNA hydrolysis by ribonuclease H and/or FEN1 (both function in removal of RNA primer during DNA replication). To try to apply this system in eukaryote would directly lead to struggle with such concerns."

(iii-2) Dr. Doudna (one of authors of Cited Document 2)

"Our research paper of 2012 was fairly successful but contained certain problem. We could not be sure whether CRISPR/Cas9 functions in eukaryote (plant and animal cells)."

(iii-3) Jinek (one of authors of Cited Document 2)

"However, it was not known whether such bacterial system functions in a eukaryotic cell."

(iii-4) Melissa Pandika

"Doudna experienced 'many setbacks' in making CRISPR function in human cells. However, she knew that, if she succeeds, CRISPR will be a ‘quite influential remarkable discovery,’ and powerful gene therapy technique."

(2) Judgment

i. Appellant's allegations i and ii

Appellant's allegation means only that there was no reasonable expectation that application of CRISPR/Cas9 to eukaryotic cells could be successful. As stated in the above 4, since there is sufficient motivation to try to cause the CRISPR/Cas system of Cited Invention 2 to function on genomes in eukaryotic cells, it cannot be deemed just because there was no reasonable expectation for its success that there was disincentive that prevents a person skilled in the art from carrying out such trial.

In addition, even if Cited Document 2 does not disclose any nuclear localization signal, regulatory element, or vector system, as stated in the above 4, as far as there is sufficient motivation to cause the CRISPR/Cas system of Cited Invention 2 functions on genomes in eukaryotic cells, it is a matter that a person skilled in the art can carry out in realizing it without special inventiveness by applying well-known arts to add a nuclear localization signal to Cas9 protein, or to introduce recombinant template or DNA that encodes chimeric RNA into a vector with DNA the same as or different from DNA that encodes Cas9 protein to create a vector system. In addition, there is no ground to recognize there was special technical difficulty in applying such well-known art.

ii. Appellant's allegation iii

While Reference Material 3 submitted by Appellant on November 1, 2017 as a supplemental statement is recognized as a ground material for the above (iii-1), the last paragraph of the material describes that "although it is not known whether the CRISPR system provides next target cleavage reagent after the next, it is surely worth it to try". This description indicates that there was motivation to apply CRISPR/Cas9 to eukaryotic cells. In addition, with respect to the description of the above (iii-1) pointed out by Appellant, there is also only a comment that there is no guarantee that CRISPR/Cas9 will function in eukaryotes.

In addition, with respect to the above (iii-2) and (iii-3) also, although the materials that are the grounds for them are not known, they only point out that no

confidence could be obtained whether CRISPR/Cas9 functions in eukaryote, or that it was not known whether it functions.

Next, with respect to the above (iii-4), Reference Material 12 submitted as a supplemental statement is recognized as the ground material. In addition, from "Doudna experienced 'many setbacks' in making CRISPR function in human cells. However, she knew that, if she succeeds, CRISPR will be a ‘quite influential remarkable discovery,’ and a powerful gene therapy technique in certain cases" it can be recognized that there was motivation to try to apply CRISPR/Cas9 to eukaryotic cells.

As explained above, what Appellant points out as contemporary evidences only point out that there is no reasonable expectation that the CRISPR/Cas system of Cited Invention 2 functions on genomes in eukaryotic cells, but, as stated in the above item i, as far as there is sufficient motivation to try to make the CRISPR/Cas system of Cited Invention 2 function on genomes in eukaryotic cells, it cannot be said just because there was no reasonable expectation of success that there was disincentive that prevent a person skilled in the art from carrying out such trial.

iii. Issue dates of Reference Documents 1 and 2 shown below are after the first priority date of the present application, but their dates of posting are before or approximately the same time as the first priority date of the present application, and those reference documents are research papers posted by multiple independent research groups other than the application of the present application. Those research papers and the originally attached description, etc. of Prior Application 1 describe cleavage of the target sequences in nucleuses in eukaryotic cells by CRISPR/Cas9 vector system in which Cas9 protein to which nuclear localization signal was added. This can be deemed to indicate that there is sufficient motivation to try to make the CRISPR/Cas system of Cited Invention 2 function on genomes in eukaryotic cells, and, even if it could not be reasonable expected before the priority date for the Invention that the CRISPR/Cas9 system functions on eukaryote, it does not constitute any disincentive, and furthermore, the use of the CRISPR/Cas9 vector system in which Cas9 protein to which nuclear localization signal is added was quite normally used by a person skilled in the art as a technique for applying the CRISPR/Cas system to eukaryotic cells. As far as no special inventiveness that exceeds the above could be found in the Invention, there is no way but to deny inventive step.

Reference Document 1: Science, Vol. 339, pp. 823-826 (Posted date: October 26, 2012; date of issue: January 3, 2013)
Reference Document 2: Life, Vol. 2, e00471, pp. 1-9 (Posted date: December 15, 2012; date of issue: January 29, 2013)

iv. As explained in the above items i to iii, Appellant's allegation cannot be accepted.

6. Summary

Accordingly, none of Applicant's allegations can be acceptable, and, since those skilled in the art could have easily invented the Invention based on the invention disclosed in Cited Document 2 and well-known arts before the priority date for the Invention, Appellant should not be granted a patent under the provisions of Article 29-2 of the Patent Act.

No. 6 Closing

As described above, Appellant should not be granted a patent for the Invention under the provisions of Article 29-2 and Article 29(2) of the Patent Act, and the present application should be rejected without examining inventions according to the other claims.

Therefore, the appeal decision shall be made as described in the conclusion.

September 14, 2018

Chief administrative judge: NAGAI, Keiko
Administrative judge: YAMANAKA, Takayuki
Administrative judge: KOGURE, Michiaki